ACID-PRODUCTION AND OTHER CHARACTERS OF BACILLUS-COLI-LIKE BACTERIA FROM FECES AND SEWAGE *

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The ability to decompose carbohydrates with the formation of acid has long been recognized as one of the characteristics of Bacillus-coli-like bacteria. This property of acid-production is the basis for the isolation of B. coli on the Wurtz litmus-lactose-agar plate, and also for the separation of B. coli from its relatives, B. typhosus and B. paratyphosus, on the Conradi-Drigalski agar medium. The ability to ferment various substances has been further utilized as a basis for the subdivision of the colon-aerogenes group. In these studies on classification, however, attention has been focused upon gas-formation rather than upon acid-production.

Browne,[1] in an extensive study of certain factors influencing acid-production, points out that Bacillus-coli-like bacteria isolated from oysters formed less acid from carbohydrates than those isolated from human stools, and he attributed the difference to a loss of fermenting power by the organisms in their passage through sewage from the intestines to the oysters. Unfortunately, this investigator did not differentiate the different types of organisms with which he was working. It is entirely probable that the smaller amount of acid observed among the oyster strains was due to a greater incidence of some particular type or species of Bacillus-coli-like microorganism rather than to a loss of fermenting power on the part of the intestinal forms. [After the completion of the experimental work for this paper, an article appeared by Clark and Lubs,[2] who pointed out that bovine fecal strains of B. coli give rise to a higher H+-ion concentration in glucose than do nonfecal (grain) strains.]

The present investigation was undertaken to determine the following:

1. Do Bacillus-coli-like organisms from different sources (particularly animal sources) give rise to different amounts of acid in the

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[1] Jour. Infect. Dis., 1914, 15, p. 580.
[2] Ibid., 1913, 17, p. 797.

decomposition of fermentable substances, and if they do, are the differences in acid-formation sufficiently great to warrant quantitative acid-production as a reliable differential index?

2. Is quantitative acid-production correlated with (a) the MacConkey types,[3] (b) the Voges-Proskauer reaction, or (c) gas-formation?

3. Are the morphologic and physiologic characteristics correlated with the source?

CULTURES STUDIED

Altogether 167 organisms were studied; 156 were obtained from sewage and from the feces of horse, cow, sheep, pig, and man, and 11 were from the collection of the American Museum of Natural History.

The method of isolation has been described in a previous paper.[4] They were all of the colon-bacillus group; that is, gram-negative, usually short rods, which formed gas from glucose and lactose, coagulated milk, and did not liquefy gelatin in 20 days.

PREPARATION OF MEDIA

The medium employed for tests of acid-production consisted of 1% peptone water to which was added 1% of the test substance. Peptone water, rather than nutrient broth, was used, to eliminate the formation of acid from traces of any other fermentable substance which might be present in beef extract or meat infusion. The reaction of the medium was neutral to phenolphthalein.

Sterilization.—The medium was tubed (10 c.c. in Durham fermentation tubes) and sterilized in the autoclave for 10 minutes at 10 pounds pressure, which is a shorter period than is recommended in the Standard Methods for Water Analysis (1912). Immediately on removal from the autoclave the medium was rapidly cooled by immersion in cold water, then incubated for 2 or 3 days at 37 C. in order to eliminate tubes which had escaped proper sterilization. Nonsterile tubes were rarely found. Sufficient medium was prepared at one time to permit a test of all the cultures on one substance. Variations in the composition of the medium were reduced to a minimum by using distilled water and the same bottle of Witte's peptone throughout the work.

DETERMINATION OF ACID-PRODUCTION

Acid-production was determined in the following manner. A tube of peptone water was inoculated from an agar-slant stock culture and incubated at the body temperature (37 C.) for 24 hours. Two standard 4-mm. loops of this 24-hour peptone culture were then inoculated into each of 2 tubes of peptone medium containing the test substance and incubated for 36 hours at 37 C. Acid-production in duplicate tubes varied so little that duplicates were not employed with dulcitol, galactose, maltose, glycerol, and salicin.

[3] Jour. Hyg., 1905, 5, p. 333; 1909, 9, p. 86.
[4] Levine: Jour. Infect. Dis., 1916, 18, p. 358.

The body temperature was selected for incubation, because, as was shown by Browne,[1] acid-production by B. coli is most rapid at this temperature. He also showed that with certain carbohydrates and alcohols the maximal amount of acid is formed in less than 24 hours. Thirty-six hours' incubation was employed for convenience in this study. With the alcohol, glycerol, and the glucosid, salicin, the 36-hour incubation period was not sufficient. Acid- and gas-formation from these substances were therefore determined after 72 hours' growth.

Titration.—As the acidity of distilled water varied on different days, the following technic was adopted in order to obviate tedious subtractions of checks. To a pail of distilled water (6 to 8 liters) was added 1% phenolphthalein solution (5 gm. phenolphthalein in 1 liter of 50% alcohol). The water was boiled vigorously for 15 minutes and then neutralized with sodium hydroxid. Of this neutral distilled water, containing the indicator, 45 c.c. were dipped out into an evaporating dish or casserole, 5 c.c. of the test culture were added, and the amount of acid determined by titration with N/20 NaOH without boiling.

TREATMENT OF RESULTS

A few extremely high or low results will influence considerably the average acid-production of a collection of organisms. The use of unqualified averages may therefore lead to a misconception of the acid-producing properties of a group. To supplement the arithmetic mean, or numeric average, some statement should be made as to the distribution of the variates about the average. This may be indicated by the probable error or by the standard deviation. The coefficient of variability (the ratio of the standard deviation to the mean) is an excellent abstract measure of variability. The modal acid-production (the amount of acid most frequently formed) may, under certain conditions, be of greater significance than the average amount of acid formed.

In this study the mean, the probable error of a single variate, the standard deviation, the coefficient of variability, and the empirical mode are employed.

The standard deviation is the measure of variability most commonly employed, particularly by mathematicians. It may be expressed mathematically as

$$\sigma = \sqrt{\frac{\Sigma d^2 f}{n}}$$

where "n" is the number of variates, or observations, "d" the deviation of the individual variates from the mean, and "f" the frequency of a deviation "d". The standard deviation serves to indicate whether the departures from the mean are

small or great. The closer the individual organisms group themselves about the mean, or average, the smaller the standard deviation.

An example may make clear the meaning and significance of the standard deviation. Suppose that the amounts of acid formed by a group (A) of 4 organisms in glucose broth are 2.1, 2.2, 2.2, and 2.3% normal acid, and that those formed by another group (B) of 4 organisms are 1.9, 2, 2.4, and 2.5% normal acid. The average for each group is 2.2, but mere inspection shows that the organisms in Group A and those in Group B are quite differently distributed with respect to this average. In large collections of data inspection is impracticable, but the standard deviation serves well in its place. The standard deviation in Group A is ± 0.07 while for Group B it is ± 0.25. The larger deviation in B denotes that the individuals in the group wander farther away from the average than do those in Group A.

The probable error is employed to indicate what confidence is to be placed in statistical results. The reliability of the mean and standard deviation may be determined by calculating their probable errors, but in this paper only the probable error of a single variate is considered. In a normal distribution the probable error of a single variate of a series of observations is defined as that departure from the mean, on either side, within which exactly one-half of the variates are found; that is, if in the study of acid-production by a large number of organisms, it is found that the mean (average) amount of acid formed is 2.25% normal, and that the probable error of a single observation is ± 0.15, then half of the organisms have formed between 2.1% and 2.4% normal acid.

The coefficient of variability is the ratio of the standard deviation to the mean ($\frac{\sigma}{M}$). It is an abstract measure of variability and may therefore be employed to advantage for comparing variability among different characters, or in the same character among different groups of organisms, particularly if their means differ widely.[5]

ACID-PRODUCTION IN SUBSTANCES FERMENTED BY ALL OF THE TEST ORGANISMS

Glucose, galactose, mannitol, maltose, and lactose were decomposed with gas-production by all strains.

A. GLUCOSE

The frequency distributions of the organisms with respect to acid-formation from glucose are shown in Table 1, where the relation of

[5] For a detailed description of these constants the reader is referred to Principles of Breeding (1907), by E. Davenport; Statistical Methods (1904), by C. B. Davenport; Precision of Measurements (1909), by Goodwin, and to an Introduction to the Theory of Statistics (1916), by Yule.

TABLE 1

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN GLUCOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19													
0.20-0.39													
0.40-0.59													
0.60-0.79				1		1					1	1	
0.80-0.99				3		3					3	3	
1.00-1.19				2		1				1	2	1	1
1.20-1.39	3	4	3	1			3	5		3	11	9	2
1.40-1.59	4	3	9	3			5	2	1	11	19	15	4
1.60-1.79	8	5	3	6	2	1	2	7	4	6	22	20	2
1.80-1.99	28	8	10	8	5	5	9	8	14	13	54	54	
2.00-2.19	10	6	17	8	10	11	1	9	5	5	41	41	
2.20-2.39	1			2	2				1		3	3	
Total acid-formers.....	54	26	42	34	19	22	20	31	25	39	156	147	9
Mode.....	1.90	1.90	2.10	2.00	2.10	2.10	1.90	2.10	1.90	1.90	1.90	1.90	1.50
Mean.....	1.85	1.77	1.84	1.71	2.03	1.76	1.70	1.79	1.91	1.71	1.80	1.82	1.46
Probable error	±.14	±.18	±.19	±.29	±.11	±.32	±.17	±.18	±.11	±.18	±.20	±.20	±.12
Standard deviation.....	±.21	±.27	±.28	±.43	±.16	±.47	±.25	±.27	±.17	±.26	±.30	±.30	±.18
Coefficient of variation....	11.3	15.2	15.2	25.1	7.9	26.7	14.7	15.1	8.9	15.2	16.6	16.5	12.3

acid-production to the source, to the MacConkey types, and to the Voges-Proskauer reaction is also indicated.

The mode for acid-production by all strains is at 1.9% normal, with the mean at 1.8% normal acid.

The means or average quantities of acid formed by the MacConkey types indicate that Types III (communior) and I (acidi-lactici) produce about equal quantities of acid (1.84 and 1.85% normal respectively), while Type II (communis) forms somewhat less (1.77%), and Type IV (aerogenes) the least amount (1.71%). A comparison of Type IV, which forms the smallest quantity of acid, with Type I, which gives the greatest amount, indicates that the means tend to exaggerate the difference between the two types in ability to form acid from glucose. Type I has a well-defined mode at 1.90% and Type IV has a very indistinct mode at about the same point. The standard deviation in Type IV is ±0.43, or three times as great as the difference between the means of the two MacConkey types. Similar observations

may be made on the other types. It is therefore apparent that quantitative acid-production in glucose is not a reliable criterion for differentiation of the MacConkey types.

There are many irregularities in the frequency distributions of organisms from different sources with respect to acid-production in glucose. The organisms from horse and man group themselves in a manner simulating a normal distribution, but the frequency curves of those from cow, sheep, and pig, contain 2 modes. These multiple modes are probably due to the choice of classes and to the small number of cultures from each source. In the other test substances multiple modes are very infrequent. In the column headed "Mode," in the frequency tables, the primary mode is recorded.

The distribution of organisms from the sheep is interesting. Two distinct groups are indicated, one of which generally produces more than 2% normal acid and the other usually less than 1%. Of the 5 low-acid-formers, 4 are from a single animal (all the cultures obtained from that animal), and they are distinguished morphologically from all the other sheep strains in that they are distinctly longer.

Among the sewage strains 2 well-defined modes are evident, at 1.9% and 1.5% normal acid, corresponding with the modes of the Voges-Proskauer-negative and the Voges-Proskauer-positive organisms respectively.

In a consideration of the different animal sources it appears that the average amount of acid formed from glucose by Bacillus-coli-like organisms from horse (2.03%) is greater than the amounts formed by strains from pig, sheep and cow (1.70, 1.76, and 1.79%), while the amount formed by human strains is intermediate (1.91% normal). This relationship does not hold for other test substances and there does not seem to be any marked relation between the quantities of acid produced from glucose, and those formed from other fermentable carbohydrates or alcohols by colon-bacillus-like organisms from the animals recorded here.

Quantitative acid-formation is better correlated with the Voges-Proskauer reaction than with the source or with MacConkey's groups. The Voges-Proskauer-negative organisms give an average of 1.82% normal acid, with a mode at 1.90%, while the Voges-Proskauer-positive strains 1.46%, with a mode at 1.50% normal, and, altho the difference, 0.36, is probably not sufficient for reliable differentiation, it is nevertheless significant, because rather striking differences in acid-formation between the Voges-Proskauer-positive and the Voges-Pros-

kauer-negative strains are observed with many other test substances, as maltose, sucrose, glycerol, and dulcitol.

B. GALACTOSE

The frequency distributions with respect to acid-production in galactose are shown in Table 2. Less acid is formed from galactose than from glucose, and the frequency distributions are very nearly normal. Multiple modes are not present. The average amount of

TABLE 2

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN GALACTOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19													
0.20-0.39				1			1				1	1	
0.40-0.59													
0.60-0.79				1						1	1	1	
0.80-0.99				2		1				1	2	1	1
1.00-1.19	3		4	6		3	1	2	1	6	13	8	5
1.20-1.39	28	18	21	10	8	6	12	17	19	15	77	76	1
1.40-1.59	21	8	16	13	11	11	6	11	5	14	58	57	1
1.60-1.79	1		1							2	2	1	1
1.80-1.99													
2.00-2.19													
Total acid-formers.....	53	26	42	33	19	21	19	30	25	39	154	145	9
Mode.....	1.30	1.30	1.30	1.50	1.50	1.50	1.30	1.30	1.30	1.30	1.30	1.30	1.10
Mean.....	1.37	1.36	1.37	1.27	1.42	1.36	1.35	1.36	1.33	1.34	1.35	1.36	1.21
Probable error	±.08	±.06	±.09	±.18	±.07	±.12	±.07	±.08	±.06	±.14	±.12	±.11	±.16
Standard deviation.....	±.12	±.09	±.13	±.27	±.10	±.18	±.11	±.12	±.09	±.20	±.17	±.16	±.23
Coefficient of variation....	8.8	6.6	9.5	21.2	7.1	13.2	8.2	8.8	6.8	14.9	12.6	11.8	19.0

acid formed by all strains is 1.35% normal, with a distinct mode at 1.30%. (Acid was not determined from 2 cultures, 1 from pig and 1 from sheep, which broke just before titration.)

The MacConkey types, I, II, and III, each have a mode at 1.30% normal acid, and means at 1.37, 1.36, and 1.37% normal acid respectively. Altho the mode for Type IV is 1.50% normal acid—somewhat higher than for the other types—the mean, 1.27%, is lower, a circumstance indicating a greater variability in Type IV. This greater

variability is indicated by the much larger standard deviation and coefficient of variability. MacConkey types, therefore, cannot be differentiated on the basis of quantitative acid-production in galactose as indicated by titration with phenolphthalein.

There does not seem to be any correlation between the amount of acid formed from galactose and the source of the organisms. One organism from the cow formed less than 0.4% acid. It was omitted in calculating acid-production by the group. If included, the mean for the cow strains becomes 1.30%, with a coefficient of variability of 19.2%.

The Voges-Proskauer-positive strains form somewhat less acid (1.21%) than do the Voges-Proskauer-negative strains (1.36%). The difference (0.15% normal) is slight, but it is greater than the differences observed with the MacConkey types or with the strains from different sources. The difference is of some interest, moreover; for, as will appear later, whereas the Voges-Proskauer-positive strains form less acid from the monosaccharids than do the Voges-Proskauer-negative strains, the reverse is true when more complex substances (except lactose) are fermented.

C. MANNITOL

The hexite, mannitol, is attacked about as readily as galactose. The average amount of acid formed by all strains is 1.32%, with a sharp mode at 1.30% normal. (Acid-production was not determined in 2 cultures, 1 from horse and 1 from man.) The frequency distributions and the relation of the Voges-Proskauer reaction, the source, and the MacConkey types to the amount of acid formed from mannitol are shown in Table 3.

The mode for each of the MacConkey types is at 1.30%, and the means are 1.32, 1.30, 1.33, and 1.31% normal acid respectively. The MacConkey types are therefore indistinguishable on the basis of quantitative acid-production from mannitol.

A comparison of the amounts of acid formed by Voges-Proskauer-negative and Voges-Proskauer-positive strains indicates that the latter attack mannitol somewhat more readily, but the difference is not appreciable.

Except for the horse strains, which have a mode at 1.50%, the organisms from all the other sources group themselves around 1.30% normal acid as a mode. In general, the differences observed between the means are too slight to be of any significance. The average of the

sheep strains is the lowest, 1.21%, as compared with 1.34% for human strains, 1.38% for horse, 1.36% for sewage, 1.31% for cow, and 1.28% normal for pig strains. The lower average of the sheep strains is due to the presence among them of a few low-acid-producing organisms rather than to a lesser ability of the group as a whole to attack mannitol. That sheep strains form acid from mannitol as readily as do those from the cow, pig, and man is indicated by the coincidence of their

TABLE 3

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN MANNITOL

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19													
0.20-0.39													
0.40-0.59													
0.60-0.79				2		2					2	2	
0.80-0.99	1			4	1	3		1			5	5	
1.00-1.19	9	6	7		1	3	5	6	3	4	22	22	
1.20-1.39	29	14	20	15	6	9	9	19	13	22	78	75	3
1.40-1.59	13	6	14	12	10	5	6	5	8	11	45	40	5
1.60-1.79	1									1	1	1	
1.80-1.99				1						1	1		1
2.00-2.19													
Total acid-formers.....	53	26	41	34	18	22	20	31	24	39	154	145	9
Mode.....	1.30	1.30	1.30	1.30	1.50	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.50
Mean.....	1.32	1.30	1.33	1.31	1.38	1.21	1.31	1.28	1.34	1.36	1.32	1.31	1.48
Probable error	±.10	±.09	±.09	±.17	±.11	±.18	±.10	±.09	±.09	±.12	±.12	±.12	±.12
Standard deviation.....	±.15	±.14	±.14	±.26	±.17	±.26	±.15	±.14	±.13	±.18	±.18	±.18	±.18
Coefficient of variation....	11.3	10.8	10.5	19.8	12.3	21.2	11.4	10.9	9.7	13.2	13.6	13.7	12.1

modes. The strains from the horse tend to form somewhat more acid than do those from other animals, but the difference is too slight to be of any differential significance.

D. LACTOSE

The amount of acid formed from the disaccharid, lactose, in 1% lactose peptone solution is very nearly the same as that formed from the monosaccharid, galactose, and from the hexite, mannitol. The mode for all strains is at 1.30%, and the mean is also at 1.30% normal acid. The frequency distributions are shown in Table 4.

MacConkey Type II has an ill-defined mode at 1.50%, and the mean is 1.25% normal acid. The mode for the other types (I, III, IV) is at 1.30%, and the means are 1.31, 1.32, and 1.33% normal acid respectively. The MacConkey types are indistinguishable on the basis of quantitative acid-production in lactose.

There is no evident relation between the source and the amount of acid produced from lactose.

TABLE 4

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN LACTOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19													
0.20-0.39		1					1				1	1	
0.40-0.59													
0.60-0.79		3					3				3	3	
0.80-0.99	1	1		6		5	1			2	8	7	1
1.00-1.19	13	1	11		4	1	1	6	12	1	25	24	1
1.20-1.39	30	9	19	15	10	3	11	18	11	20	73	67	6
1.40-1.59	8	11	10	10	5	9	8	7	2	13	39	38	1
1.60-1.79			2	3		4				1	5	5	
1.80-1.99	2									2	2	2	
2.00-2.19													
Total acid-formers.....	54	26	42	34	19	22	20	31	25	39	156	147	9
Mode.....	1.30	1.50	1.30	1.30	1.30	1.50	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Mean.....	1.30	1.25	1.31	1.32	1.31	1.25	1.16	1 31	1.22	1.38	1.30	1.31	1.26
Probable error	±.12	±.22	±.11	±.16	±.09	±.19	±.22	±.09	±.09	±.13	±.15	±.15	±.11
Standard deviation.....	±.17	±.32	±.17	±.23	±.14	±.28	±.33	±.13	±.13	±.20	±.22	±.22	±.16
Coefficient of variation....	13.1	25.6	13.0	17.4	10.7	22.4	28.4	10.0	10.6	14.5	16.9	16.8	12.7

There is no distinction between the amounts of acid produced from lactose by Voges-Proskauer-positive and Voges-Proskauer-negative strains. Both groups have their modes at 1.30%, and the means are but slightly removed from the modes, being 1.26 and 1.31% normal acid respectively.

E. MALTOSE

Decomposition of the disaccharid, maltose, yields considerably less acid than the decomposition of the monosaccharids, glucose and galactose, the hexite, mannitol, or the disaccharid, lactose, mentioned. All

strains considered, the average acid-production was 0.77%, with a very distinct mode at 0.7% normal acid. The frequency distributions of the organisms from different sources, of the MacConkey types, and of the Voges-Proskauer reaction with respect to acid-production in maltose are shown in Table 5. One organism apparently fails to show acid but forms gas. This neutral reaction is presumed to be due to reversion.

TABLE 5

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN MALTOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19		1								1	1	1	
0.20-0.39													
0.40-0.59	7	2	6	1		3		3	6	4	16	16	
0.60-0.79	31	17	24	18	9	19	13	15	18	16	90	90	
0.80-0.99	10	6	8	9	9		3	12	1	8	33	31	2
1.00-1.19	6		2	4	1		4	1		6	12	8	4
1.20-1.39			2	2						4	4	1	3
1.40-1.59													
1.60-1.79													
1.80-1.99													
2.00-2.19													
Total acid-formers.....	54	25	42	34	19	22	20	31	25	38	155	146	9
Mode.....	0.70	0.70	0.70	0.70	0.80	0.70	0.70	0.70	0.70	0.70	0.70	0.70	1.10
Mean.....	0.76	0.73	0.76	0.83	0.81	0.67	0.81	0.77	0.67	0.85	0.77	0.75	1.12
Probable error	±.11	±.07	±.13	±.13	±.08	±.05	±.11	±.09	±.08	±.15	±.11	±.09	±.10
Standard deviation.....	±.16	±.11	±.19	±.19	±.11	±.07	±.16	±.14	±.12	±.23	±.17	±.14	±.15
Coefficient of variation....	21.0	15.1	25.0	22.9	13.6	10.4	19.8	18.2	17.9	27.0	22.1	18.7	13.4

Each of the MacConkey types has a sharply defined mode at 0.70% normal. The means for the types are 0.76, 0.73, 0.76, and 0.83% normal acid respectively. There is no correlation between quantitative acid-formation from maltose and the MacConkey types.

The relation of acid-formation from maltose to the source of Bacillus-coli-like organisms is not at all striking. The mode for each source is at 0.7% normal acid. The trend of the variation among the strains from horse, cow, pig, and sewage, is beyond the mode, so that the means are 0.81, 0.81, 0.77, and 0.83% normal acid respec-

tively, while the strains from man and sheep vary in the other direction, lowering the averages to 0.67% for man and 0.68% for sheep. It should be pointed out that the relatively high average for sewage, 0.83%, is due to the presence of Voges-Proskauer-positive organisms. The average for the sewage strains exclusive of the Voges-Proskauer-positive organisms, is 0.73% normal acid. Acid-production in maltose can not be considered a reliable index for differentiation of Bacillus-coli-like organisms from the sources studied.

There is a rather marked and distinct relation between quantitative acid-production in maltose and the Voges-Proskauer reaction. It appears from Table 5 that the Voges-Proskauer-negative strains occasionally form more than 1% acid, but usually less than 0.8%, while the Voges-Proskauer-positive strains usually form more than 1% and never less than 0.8% normal acid. The mode for the Voges-Proskauer-negative strains is at 0.70% and the mean at 0.78% normal acid. The mode and mean for the Voges-Proskauer-positive strains are 1.10 and 1.12% respectively.

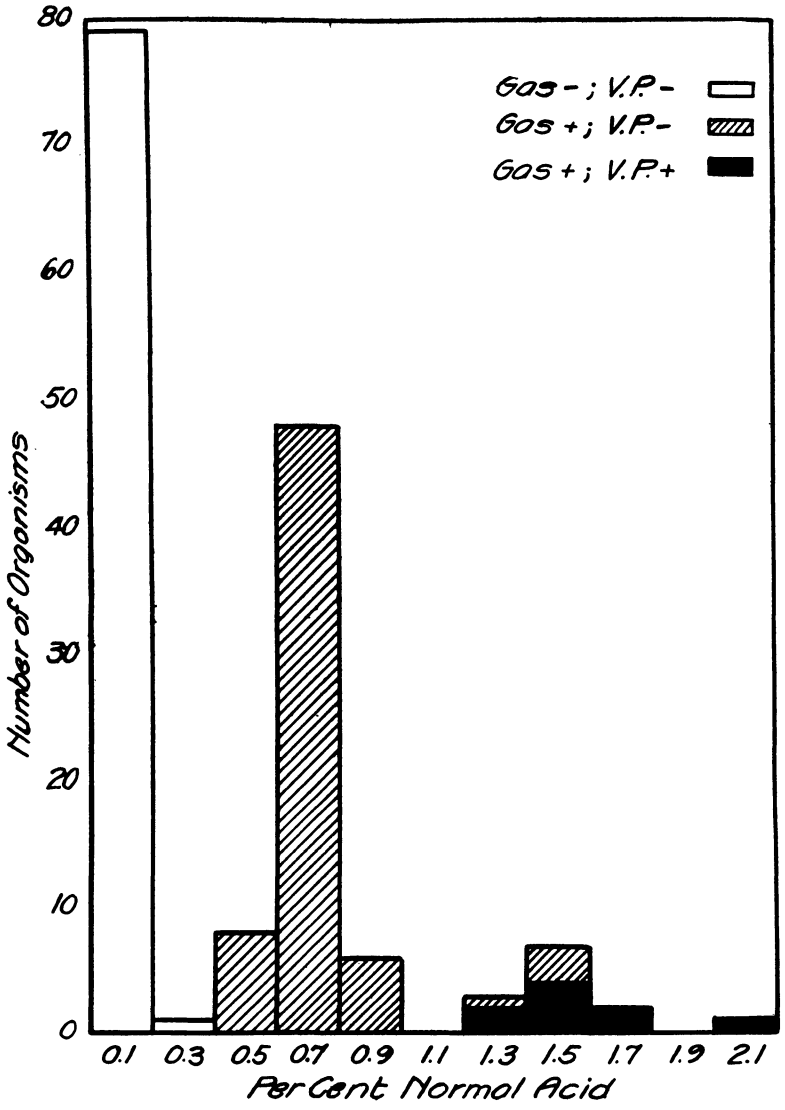
ACID-PRODUCTION IN SUBSTANCES NOT FERMENTED BY ALL THE TEST ORGANISMS

The disaccharid, sucrose, the trisaccharid, raffinose, the glucosid, salicin, and the alcohols, glycerol and dulcitol, were attacked by many, but not by all, of the organisms studied.

In calculating means and other constants for acid-formation, only those organisms which attacked the test substances were included. The line of demarcation for acid-production was selected at 0.4% normal acid, because in sucrose, raffinose, and dulcitol organisms which formed less than this amount in 36 hours at 37 C. rarely, if ever, formed gas, while those which produced more than 0.4% acid, practically always formed gas also.

A. SUCROSE

In Table 6 are shown the frequency distributions of acid-production in sucrose in relation to the MacConkey types, the source, and the Voges-Proskauer reaction. The relation of acid-production to gas-formation, and to the Voges-Proskauer reaction, is indicated, also, in Plot 1. (One organism, which was overrun in titration, is not included in the calculation.) Three modes are evident. One mode is at 0.1% normal acid and represents those organisms which do not form gas from sucrose. Acid-formation and gas-production in suc-



PLOT I

ACID PRODUCTION FROM SUCROSE (All Strains)

rose are well correlated. Colon-bacilli-like organisms which form gas, also give rise to acid and vice versa. Among the gas-formers 2 groups are apparent; one forms acetylmethylcarbinol from glucose (V.P.+) and a relatively large amount of acid from sucrose (mode at 1.50% normal), while the other does not form acetylmethylcarbinol from glucose (V.P.—) and gives rise to a much smaller quantity of acid from sucrose (an extremely sharp and distinct mode at 0.70% normal).

MacConkey Types I and II do not form acid from sucrose. That Types III and IV are indistinguishable on the basis of quantitative acid-production in sucrose, is apparent from Table 6.

Ten of the organisms from cow, 15 from horse, 20 from sheep, 10 from pig, and only 3 from man, ferment sucrose. The amount of acid formed bears no definite relation to the animal source, but it should

TABLE 6

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN SUCROSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19	53	26			4	1	10	21	21	22	79	79	
0.20-0.39	1								1		1	1	
0.40-0.59			3	5		3		2		3	8	8	
0.60-0.79			30	18	10	16	9	7	3	3	48	48	
0.80-0.99			4	2	2	1	1	1		1	6	6	
1.00-1.19													
1.20-1.39			1	2	1					2	3	1	2
1.40-1.59			3	4	2					5	7	3	4
1.60-1.79			1	1						2	2		2
1.80-1.99													
2.00-2.19				1						1	1		1
Total acid-formers.....	....	....	42	33	15	20	10	10	3	17	75	66	9
Mode.....	....	....	0.70	0.70	0.70	0.70	0.70	0.70	0.70	1.50	0.70	0.70	1.50
Mean.....	....	....	0.80	0.91	0.93	0.68	0.72	0.68	0.70	1.18	0.84	0.74	1.57
Probable error			±.18	±.27	±.20	±.06	±.04	±.07		±.33	±.23	±.14	±.16
Standard deviation.....			±.27	±.40	±.29	±.09	±.06	±.11		±.49	±.34	±.20	±.23
Coefficient of variation....	....	....	33.8	44.0	31.2	13.2	8.3	16.2		41.5	40.5	27.0	14.6

be noted that a few cultures among the horse strains form considerably more acid than any of the other animal strains. The high average for

the horse strains is due to the influence of these few cultures and is not a characteristic of horse strains in general.

The high average, 1.18% normal acid, of the 17 sewage strains which attacked sucrose, is due entirely to the presence among them of 9 Voges-Proskauer-positive organisms. The mean for the other 8 sewage strains is 0.75% normal acid.

Voges-Proskauer-negative strains attack sucrose less readily than the Voges-Proskauer-positive strains. The means for the two groups are 0.74 and 1.57%, and the empirical modes 0.7 and 1.5% normal acid respectively.

B. RAFFINOSE

The frequency distributions of the organisms with respect to acid-production in raffinose and the relation of acid-formation to the MacConkey types, to the source, and to the Voges-Proskauer reaction, are given in Table 7.

TABLE 7

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN RAFFINOSE

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19	50	23			3		8	20	21	21	73	73	
0.20-0.39		1					1				1	1	
0.40-0.59	1		2	1	1	1		1		1	4	4	
0.60-0.79			13	5	5	4	2	5		2	18	18	
0.80-0.99	2	1	10	5	3	3	6	2	2	2	18	18	
1.00-1.19	1		8	11	4	8	1	2	1	4	20	19	1
1.20-1.39		1	8	10	3	5	2	1	1	7	19	13	6
1.40-1.59			1	2		1				2	3	1	2
1.60-1.79													
1.80-1.99													
2.00-2.19													
Total acid-formers.....	4	2	42	34	16	22	11	11	4	18	82	73	9
Mode.....	....	....	0.70	1.10	0.70	1.10	0.90	0.70	0.90	1.80	1.10	1.10	1.80
Mean.....	....	....	0.95	1 08	0.94	1.03	0.97	0.85	1.05	1.11	1.00	0.96	1.32
Probable error			±.17	±.17	±.15	±.17	±.12	±.15	±.11	±.19	±.17	±.16	±.08
Standard deviation.....			±.25	±.25	±.22	±.25	±.18	±.22	±.17	±.28	±.26	±.24	±.11
Coefficient of variation....	....	....	26.3	22.9	23.4	24.3	18.6	25.9	16.2	25.2	26.0	25.5	8.3

All the strains considered, somewhat more acid is formed from raffinose (1% normal) than from sucrose (0.7%). No distinct mode

is present, and the dispersion of the distribution is very great, as indicated by a large coefficient of variability (26%).

MacConkey Types I and II generally do not attack raffinose. The few strains in these types which do, are not sufficient for comparative purposes. Type IV tends to form more acid than Type III, but the difference is not considered a reliable index for differentiation.

There is no apparent relation between the animal source and the amount of acid formed from raffinose. The mean for the sewage strains, 1.11% normal acid, is higher than for those from other sources. This difference, as was observed in the case of sucrose, is due to the presence of the Voges-Proskauer-positive group among the sewage strains. The mean for the Voges-Proskauer-negative strains in sewage is 0.94% normal acid.

The Voges-Proskauer-negative strains attack raffinose less readily than do the Voges-Proskauer-positive strains. The means for the two groups are 0.96 and 1.32% normal acid respectively, but the variability among the strains is such as to make the difference (0.36) of questionable significance.

C. GLYCEROL

The alcohol, glycerol, is attacked by many strains which form acid but not gas. For calculating acid-production all strains which form more than 0.4% normal acid in 72 hours at 37 C. are regarded as acid-formers. The frequency distributions and the relation of quantitative acid-production to the MacConkey types, to the source, and to the Voges-Proskauer reaction are shown in Table 8. A sharp mode is observed at 0.70% and the mean for all the strains is at 0.73% normal acid.

The MacConkey types are indistinguishable on the basis of quantitative acid-production in 1% glycerol peptone solution altho Type III tends to form somewhat less acid than the others.

The differences between the means of organisms from the various animal sources cannot be regarded as significant. The sheep strains form the least amount of acid (0.60% normal), while the means for strains from other sources are, horse 0.67%, cow 0.71%, man 0.71%, and pig 0.76% normal acid. The somewhat higher mean of the sewage strains (0.83%) is due, again, to the influence of Voges-Proskauer-positive strains. These eliminated, the mean for the Voges-Proskauer-negative strains in sewage is 0.70% normal.

One of the Voges-Proskauer-positive strains does not attack glycerol. This is probably B. cloacae. Those Voges-Proskauer-positive organisms which do ferment glycerol, generally form much more acid than the Voges-Proskauer-negative fermenting strains. The mean for the Voges-Proskauer-negative organisms coincides with the mode at 0.70% normal. The Voges-Proskauer-positive organisms have a mode at 1.50%, with a mean at 1.28% normal acid.

TABLE 8

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN GLYCEROL

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19		1	1	2	1					3	4	3	1
0.20-0.39	2		2	1		2		2		1	5	5	
0.40-0.59	10	1	20	8	8	10	2	4	7	8	39	39	
0.60-0.79	25	16	13	12	5	9	15	13	13	11	66	66	
0.80-0.99	16	8	3	6	5		3	12	3	10	33	31	2
1.00-1.19	1			1					1	1	2	1	1
1.20-1.39				2					1	1	2	1	1
1.40-1.59			3	1						4	4		4
1.60-1.79													
1.80-1.99													
2.00-2.19													
Total acid-formers.....	52	25	39	30	18	19	20	29	25	35	146	138	8
Mode.....	0.70	0.70	0.50	0.70	0.50	0.50	0.70	0.70	0.70	0.70	0.70	0.70	1.50
Mean.....	0.73	0.76	0.67	0.77	0.67	0.60	0.71	0.76	0.71	0.83	0.73	0.70	1.28
Probable error	±.10	±.07	±.17	±.17	±.11	±.06	±.07	±.09	±.13	±.20	±.14	±.11	±.16
Standard deviation.....	±.15	±.10	±.25	±.25	±.17	±.09	±.10	±.14	±.19	±.30	±.21	±.16	±.24
Coefficient of variation....	20.5	13.2	37.4	32.5	25.4	15.0	14.1	18.4	26.8	36.2	28.8	24.3	16.0

D. DULCITOL

In Table 9 is indicated the relation of the MacConkey types, the source, and the Voges-Proskauer reaction to acid-production in dulcitol.

MacConkey Types I and IV do not form acid from dulcitol. Types II and III produce about equal quantities, 0.88% and 0.81% normal acid respectively, but Type II shows a greater variability.

The sheep, pig, human, and horse strains attack dulcitol more vigorously than do the organisms from the cow. The averages are,

TABLE 9

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN DULCITOL

Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19	53			33	6	11	9	18	19	23	86	80	6
0.20-0.39		1	1	1	2		1				3	3	
0.40-0.59	1	5				1	2		1	2	6	6	
0.60-0.79		7	16		5	1	5	4	2	6	23	23	
0.80-0.99		6	21		6	9	3	4	1	4	27	27	
1.00-1.19		6	1					5	2		7	7	
1.20-1.39			3							3	3		3
1.40-1.59													
1.60-1.79													
1.80-1.99													
2.00-2.19													
Total acid-formers.....	1	24	41		11	11	10	13	6	15	66	63	3
Mode.....	....	0.70	0.90		0.90	0.90	0.70	1.10		0.70	0.90	0.90	1.30
Mean.....	....	0.81	0.88		0.81	0.84	0.72	0.92	0.83	0.85	0.83	0.81	1.30
Probable error		±.15	±.13		±.07	±.08	±.09	±.11	±.15	±.18	±.15	±.11	
Standard deviation.....		±.22	±.20		±.10	±.12	±.14	±.16	±.22	±.26	±.22	±.17	
Coefficient of variation....	....	27.2	22.7		12.3	14.3	19.4	17.4	26.5	30.6	26.5	21.0	

pig 0.92%, sheep 0.84%, human 0.83%, and horse 0.81%, as compared with 0.72% normal acid for cow. The number of fermenting strains from the different sources is too small for reliable comparison and the differences here indicated are insignificant.

Only 3 of the Voges-Proskauer-positive strains ferment dulcitol, but the amount of acid produced by each of these three organisms is greater than that formed by any of the Voges-Proskauer-negative strains. The mean for the Voges-Proskauer-positive organisms is 1.30%, and for the Voges-Proskauer-negative cultures 0.81% normal acid.

E. SALICIN

Acid-production in salicin, as in glycerol, is not always accompanied by gas-formation. The frequency distribution with respect to source, MacConkey type, and Voges-Proskauer reaction is indicated in Table 10.

TABLE 10

RELATION OF SOURCE, MACCONKEY TYPE, AND VOGES-PROSKAUER REACTION TO ACID-PRODUCTION IN SALICIN

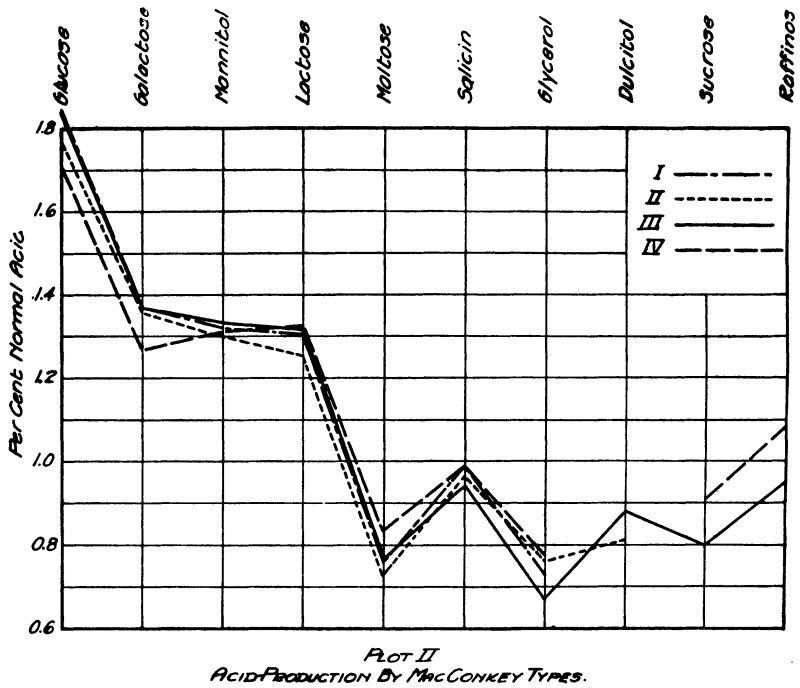
Percentage of Normal Acid	Frequencies												
	MacConkey Type				Source						All Strains	Voges-Proskauer	
	I	II	III	IV	Horse	Sheep	Cow	Pig	Man	Sewage		Negative	Positive
0.00-0.19	26	1	8	8	6	7	1	8	13	8	43	43	
0.20-0.39				1			1				1	1	
0.40-0.59	1			3				2	1	1	4	3	1
0.60-0.79	6	5	10	4	3	6	3	5	4	4	25	25	
0.80-0.99	8	9	16	7	5	6	10	7	5	7	40	40	
1.00-1.19	7	9	4	5	2	3	3	7	2	8	25	23	2
1.20-1.39	6	2	1	4	3		2	2		6	13	11	2
1.40-1.59			2	1						3	3		3
1.60-1.79				1						1	1		1
1.80-1.99			1							1	1	1	
2.00-2.19													
Total acid-formers.....	28	25	34	25	13	15	18	23	12	31	112	103	9
Mode.....	0.90	1.00	0.90	0.90	0.90	0.90	0.90	1.00	0.90	1.10	0.90	0.90	1.50
Mean.....	0.98	0.96	0.94	0.98	0.98	0.94	0.94	0.92	0.83	1.11	0.96	0.94	1.28
Probable error	±.16	±.12	±.17	±.21	±.15	±.07	±.12	±.15	±.12	±.21	±.16	±.15	±.22
Standard deviation.....	±.23	±.17	±.26	±.30	±.22	±.11	±.17	±.22	±.17	±.31	±.23	±.22	±.33
Coefficient of variation....	23.5	17.7	27.7	30.6	22.5	11.7	18.1	23.9	20.5	27.9	23.9	23.4	25.8

Inspection of Table 10 shows that the MacConkey types cannot be differentiated on the basis of the amount of acid formed from salicin. Neither is quantitative acid-production an index to the animal source. The Voges-Proskauer-positive strains give more acid (1.28% normal) than the Voges-Proskauer-negative strains (0.94% normal), but the difference is not as marked or distinct as with sucrose and should not be regarded as a differential index.

RÉSUMÉ

A study of the quantities of acid formed by Bacillus-coli-like organisms from different sources (pig, cow, sheep, horse, man, and sewage) when they are inoculated into peptone water containing 1% of various fermentable substances, indicates the following:

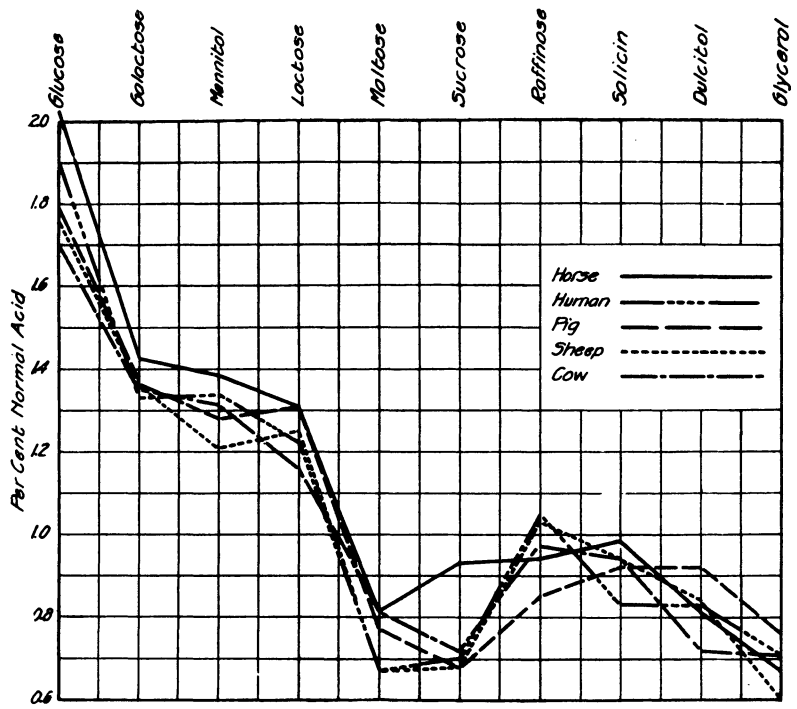
1. The MacConkey types are indistinguishable on the basis of quantitative acid-production in the fermentable carbohydrates, the alcohols, and the glucosid studied. This is shown in Plot II, in which



the curves for the different types run almost parallel and very close together.

2. That there is no correlation between the amount of acid formed from the carbohydrates, the alcohols, and the glucosid studied, and the animal source, is apparent from Plot III, in which a parallelism similar to that in Plot II is observed. The high average of acid-formation in sucrose among the horse strains is due to the presence among them of a few high-acid-producing cultures, not to any ability of horse strains, as a whole, to yield more acid from sucrose. This has been previously indicated in Table 6.

3. In a general way, the Voges-Proskauer-positive strains isolated from sewage form less acid from the monosaccharids, but more acid from the more complex carbohydrates, etc., (except lactose) than the Voges-Proskauer-negative strains. That this difference is not peculiar to the strains isolated for this study, but is characteristic of the Voges-Proskauer-positive and -negative strains in general, is indicated by the



PLOT III

ACID-PRODUCTION BY COLI-LIKE BACTERIA FROM ANIMAL FECES

similar results obtained with the 11 cultures from the collection of the American Museum of Natural History. Four of the museum strains were positive and 7 negative for the Voges-Proskauer reaction.

The average quantities of acid formed from different substances by the Voges-Proskauer-positive and -negative strains obtained from the museum collection and isolated in this laboratory are shown in Table 11 and Plot IV.

The museum strains, both Voges-Proskauer-positive and -negative, form less acid from lactose than the organisms freshly isolated from animals and sewage, but in all other substances tested the differences between the museum and freshly isolated strains are inappreciable. To infer that the museum strains have lost their power to ferment lactose does not offer an adequate explanation of all the phenomena, for it becomes necessary to explain why the organisms should single out and taboo lactose while retaining their power to form acid from the simpler and more easily attacked monosaccharids, as well as the more

difficultly fermented disaccharids, trisaccharid, alcohols, and glucosid studied. No attempt will therefore be made to explain this phenomenon with lactose, except to suggest that it may possibly be attributed to the small number of museum strains studied.

An inspection of Plot IV and Table 11 indicates that all the 167 strains studied considered, the Voges-Proskauer-positive organisms form less acid from glucose than do the Voges-Proskauer-negative strains, and about equal quantities from galactose, mannitol, and lactose. In all other test substances—maltose, salicin, raffinose, dulcitol, glycerol, and sucrose—the Voges-Proskauer-positive strains give rise to more acid, the excess increasing in the order named. The differ-

TABLE 11

ACID-PRODUCTION IN FERMENTABLE SUBSTANCES BY VOGES-PROSKAUER-POSITIVE AND -NEGATIVE BACILLUS-COLI-LIKE BACTERIA

Test Substance	Percentage of Normal Acid				Excess of Acid (in Percentage of Normal) by the V. P.+ Organisms	
	American-Museum Strains		Levine's Strains			
	V. P. —	V. P. +	V. P. —	V. P. +	American-Museum Strains	Levine's Strains
Glucose	1.82	1.52	1.82	1.46	— .30	— .36
Galactose	1.31	1.28	1.36	1.21	— .03	— .15
Lactose	0.96	0.85	1.31	1.26	— .11	— .05
Mannitol	1.37	1.41	1.31	1.48	+ .04	+ .17
Maltose	0.66	1.01	0.75	1.12	+ .35	+ .37
Sucrose	0.71	1.52	0.74	1.57	+ .81	+ .83
Raffinose	0.79	1.22	0.96	1.32	+ .43	+ .36
Glycerol	0.58	1.27	0.70	1.28	+ .69	+ .58
Dulcitol	0.83	1.15	0.81	1.30	+ .32	+ .49
Salicin	1.00	1.38	0.94	1.28	+ .38	+ .34

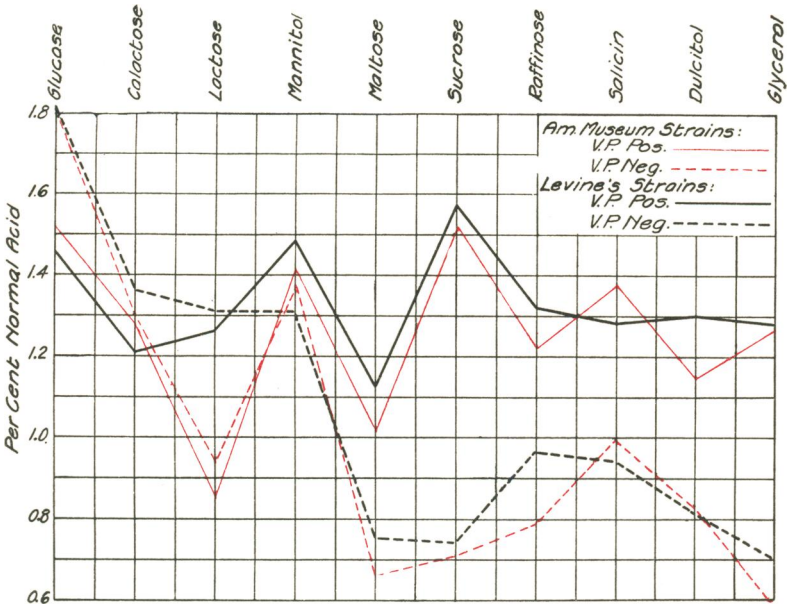
ences obtained in salicin, raffinose, and possibly glucose, are probably not significant, on account of the variations observed in acid-production from these substances.

THE SUPPOSED LOSS OF FERMENTING POWER BY B. COLI IN ITS PASSAGE THROUGH SEWAGE

Browne observed that colon-bacillus-like organisms from oysters formed less acid from glucose than did similar organisms derived from man. He concludes: "The bacillus coli isolated from feces, both from laboratory assistants and from the immigrants of the *S. S. Roma,* produced more acid in dextrose and lactose broth than the colon bacillus isolated from oysters. This seems to indicate that Bacillus coli loses some of its ability to ferment carbohydrate with the production

of acid during the journey from the intestinal tract to the oysters." He states, however, that in his laboratory experiments he was unable to cause a reduction in fermenting power even after long periods (8 weeks) of storage in sea water.

It appears from this study that a very plausible explanation of Browne's results is that Voges-Proskauer-positive organisms were among his oyster strains. Such organisms are very rare in feces, but not uncommon in sewage and soil washings. The admixture of a



PLOT IV
Acid-Production by Voges-Proskauer Positive and Negative Coli-Like Bacteria

few Voges-Proskauer-positive organisms in a collection of colon-bacillus-like strains would decrease the mean amount of acid formed from glucose and raise the titer of that from sucrose. The oyster strains employed by Browne formed less acid from glucose, and somewhat more from sucrose, than the fecal strains, thus confirming to some extent the inference that the differences he observed were due to an admixture of a few Voges-Proskauer-positive organisms rather than to a loss of fermenting power by colon-bacillus-like strains in their passage through sewage.

THE SUBSTITUTION OF QUANTITATIVE ACID-PRODUCTION FOR GAS-FORMATION AS A DIFFERENTIAL INDEX IN STUDIES ON B. COLI

Kligler[6] suggests that quantitative acid-production be substituted for gas-formation as an index of fermentation. He points out that in standard meat-infusion sugar-freed carbohydrate broth media there is a rather sharp dividing line between acid-producers and nonacid-producers at 1.5% normal acid, and that quantitative gas-production is variable and unreliable. Of course it is agreed that, as a quantitative test, gas-formation as ordinarily determined in the Smith or Durham tube is of little value; as a qualitative test, however, it may be of considerable significance. If a culture is inoculated into sugar broth and gas is formed, while no gas is produced in plain broth, the organism would most certainly be regarded as a fermenter irrespective of whether more or less than 1.5% acid is formed.

Kligler apparently regards such an organism as a nonfermenter, for he says: "The members of the proteus group, on the other hand, produced from 10 to 20 per cent. gas in lactose broth tho at no time did they produce more than 1.0 percent normal acid," and he later records this group as lactose-negative. It is not the intention to debate at this point whether B. proteus is a lactose-fermenter or not, but it should be pointed out that to say that an organism which forms gas from a carbohydrate is a nonfermenter because the acid titer is low, introduces confusion into the already much maligned and abused term "fermentation." The low titer might be due to a secondary alkali-production which masks the acid, as suggested by Rogers. It has been repeatedly observed in this laboratory that B. aerogenes in peptone dipotassium-phosphate solution containing 1 or 2% glucose, may be acid to methyl red after 24 hours' incubation, but alkaline after from 48 to 96 hours at 37 C.

Rogers, Clark, and Evans[7] also determined titratable acid and selected 1% normal acid as the point of demarcation between fermenters and nonfermenters, but they point out the possible errors in acid-determination and give precedence to gas-formation as indicated in the following:

"Under certain circumstances which have not yet been definitely determined, the acid from the fermentation of sugar may be masked by a secondary alkaline production, sufficient in some cases entirely to obscure the acid for-

[6] Jour. Infect. Dis., 1914, 15, p. 137.

[7] (a) Jour. Infect. Dis., 1914, 14, p. 411; (b) 15, p. 100; (c) 1915, 17, p. 137.

mation. In one small group of this collection, the lactose broth tubes at the end of seven days were only slightly more acid than the blank, altho all of the cultures gave gas in lactose bile. In no case was the titration of the culture less than that of the blank, altho this was usually the case with broths in which there was no fermentation. Where positive evidence of the fermentation of a sugar was obtained in another way, the negative evidence of the titration was disregarded and in the correlation tables the culture was included with the positive reactions. If, for instance, the titration of lactose broth was negative, while the lactose bile fermentation tubes showed gas, the cultures were considered to be lactose positive."

Acid-production should not be given precedence over gas-formation. They may be independent characters. If however, after careful studies, it appears that there is a marked correlation between quantitative acid-production and qualitative gas-formation, then it may be feasible to supplement, if not substitute, the gas test by the acid test. In that event, the line of demarcation between fermenters and non-fermenters would have to be determined for the medium employed. In this study, with peptone water containing 1% carbohydrate, non-fermenters rarely produced as much as 0.2% normal acid.

Another point of disagreement as to acid-production by B. coli is the maximal amount of acid formed. Kligler,[6] using meat-infusion media, often obtained titers of 4% normal acid or more, and similar results have been recorded by Rogers.[7] Browne,[1] however, using Liebig's meat-extract media, states that the limiting acidity for B. coli is 2.4% normal acid as determined by titration with phenolphthalein. Winslow and Walker[8] determined the acid-production in 12 substances by B. coli. The maximal acidity observed was 0.45 c.c. N/20 NaOH to the cubic centimeter of culture medium, or 2.25% normal acid.

In the study recorded here, with peptone water as the basic medium, the results are in entire accord with Winslow and Walker's, and with Browne's. Of more than 2500 titrations, none showed more than 2.4% normal acid.

The difference in acid-production observed by various investigators is probably due to differences in the composition of the media employed. It is now well established that more acid is formed in meat-infusion broth than in beef-extract broth. In media containing much phosphates, as yeast water, even more acid is formed than in meat-infusion broth. Within certain limits the amount of acid formed, as determined by titration with phenolphthalein, is a function of the amount of buffer substances (as K_2HPO_4 amino-acids, extractives, etc.) present in the culture medium. Acid is formed until a certain

[8] Science, 1907, 26, p. 797.

H+-ion concentration is reached. The ratio of the total titratable acid formed to the maximal or limiting H+-ion concentration is not constant, but varies, within limits, with the amount of buffer materials present in the medium.

The limiting H+-ion concentration may be an index of (1) the resistance of an organism to acid (H+ ions), or (2) the point of equilibrium between the decomposing carbohydrate and its end products under the influence of an organism.

If the limiting H+-ion concentration in glucose broth is such as to inhibit further growth of the organism, then the organism will die and the H+-ion concentration will remain constant. This seems to be the course of events with the Voges-Proskauer-negative group. With the Voges-Proskauer-positive organisms the H+-ion concentration rises to a maximum and then decreases, the medium becoming alkaline to methyl red. Under these conditions it is inferred that the maximal H+-ion concentration is a measure of the point of equilibrium between glucose and its end products under the influence of the organism in question.

It may be further considered that after the limiting H+-ion concentration is reached, the organism, if not destroyed, will, if capable, attack the peptones forming alkali. Some of the free acid becomes neutralized and more carbohydrate may be decomposed. The H+-ion concentration would remain constant as long as there is any fermentable carbohydrate present. If this assumption is correct, then an increase of the carbohydrate should retard the reversion from an acid to an alkaline reaction. This is exactly what takes place. In some work now in progress it has been found that Voges-Proskauer-positive strains were alkaline to methyl red after 24 hours' incubation in 0.5% peptone dipotassium-phosphate solution containing 0.5% glucose. In the same medium with 1% glucose, the reaction was acid after 24 hours but alkaline after from 48 to 72 hours. With 2% glucose, the acid reaction persisted until the 4th or 5th day. With 5% glucose there was no reversion to an alkaline reaction even after several weeks.

THE CORRELATION OF ACID- AND GAS-FORMATION

Table 12 shows the relation of gas-production to the amount of acid formed from sucrose, raffinose, dulcitol, glycerol, and salicin. The other test substances are not indicated because they were invariably fermented with production of gas. Cultures are regarded as gas-

formers if gas is observed in the closed arm irrespective of the quantity.

Table 12 indicates that with sucrose, raffinose, and dulcitol, acid- and gas-production after 36 hours' incubation at 37 C. are strikingly correlated. Of 80 organisms which fail to form gas from sucrose, 79 (98.8%) form less than 0.2% normal acid, and the remaining culture forms only 0.2% normal acid. Among the 75 organisms which do give gas from sucrose, 8 (10.6%) form more than 0.4 but less than 0.6%, 48 (64%) give between 0.6 and 0.79%, and the remaining 19 strains (25.4%) form more than 0.8% normal acid. There is no overlapping whatever between the amounts of acid produced by the gas-formers and the nongas-formers. To summarize: of the 80 strains which fail to produce gas, none form more than 0.2% normal acid, while among the 74 gas-producers the minimal amount of acid produced in peptone solution containing 1% sucrose is more than 0.4% normal acid.

A similar correlation is observed between acid- and gas-formation in 1% dulcitol in peptone solution. Of 88 strains that do not form gas, 86 (97.8%) give less than 0.2% normal acid. The remaining two organisms form 0.3% and 0.4% normal acid. Among the 67 gas-formers, however, there are only 2 (3%) that produce less than 0.4% normal acid.

The correlation of acid- and gas-production in peptone raffinose solution is also very marked; 79 produce gas and 77 fail to form gas from raffinose. Of the nongas-formers 72 (93.5%) form less than 0.2% normal acid; 3 organisms (3.9%) between 0.2% and 0.6% acid; and 2 cultures (2.6%) more than 0.8% acid. Among the gas formers 1 culture (1.3%) produces no acid, while 2 others (2.5%) form less than 0.6% normal acid. The other 76 gas-formers (96.2%) form more than 0.6% normal acid.

With glycerol and salicin the correlation of acid-production and gas-formation is not nearly so striking as it is with sucrose, dulcitol, or raffinose.

Gas is formed from glycerol by 118 of the cultures after 72 hours' incubation, while 38 organisms do not form gas. Of the gas-formers, 16 (13.6%) produce 0.4-0.59% normal acid as compared with 23 (60.6%) of the nongas-formers, while 61 (51.7%) of the former and 5 (13.2%) of the latter give 0.6-0.79% normal acid. One organism which does not form gas yields more than 0.8% normal acid.

TABLE 12

RELATIONSHIP BETWEEN QUANTITATIVE ACID-PRODUCTION AND GAS-FORMATION BY B. COLI

Test Substance	Gas		Percentage or Normal Acid				
			0-0.19	0.20-0.39	0.40-0.59	0.60-0.79	0.80 or more
Sucrose	+	No.	0	0	8	48	19
		%			10.6	64.0	25.4
	−	No.	79	1	0	0	0
		%	98.8	1.2			
Raffinose	+	No.	1	0	2	18	58
		%	1.3		2.5	22.8	73.4
	−	No.	72	1	2	0	2
		%	93.5	1.3	2.6		2.6
Dulcitol	+	No.	0	2	5	23	37
		%		3.0	7.5	34.3	55.2
	−	No.	86	1	1	0	0
		%	97.8	1.1	1.1		
Salicin	+	No.	0	0	1	19	82
		%			10	18.6	80.4
	−	No.	43	1	3	6	1
		%	79.7	1.8	5.6	11.1	1.8
Glycerol	+	No.	0	0	16	61	41
		%			13.6	51.7	34.7
	−	No.	4	5	23	5	1
		%	10.5	13.2	60.5	13.2	2.6

Salicin is fermented, with gas-formation, by 102 organisms after 72 hours at 37 C., while 54 strains do not form gas. Among the nongas-formers, 10 (18.5%) produce 0.4-0.8% normal acid, whereas this quantity of acid is also formed by 20 (19.6%) of the gas-formers.

It appears from Table 12 that under the conditions of these experiments, acid-production in sucrose, dulcitol, and raffinose is well correlated with the presence or absence of gas. With salicin the correlation is not so marked, while with glycerol the line of demarcation between gas-formers and nongas-formers, as indicated by the quantity of acid produced, is very indistinct. The substitution of quantitative acid-production for gas-formation would therefore be particularly undesirable when working with glycerol.

These results are well in accord with those of Winslow and Walker,[8] who observe: "Gas-formation coincided with acidity except in the case of dextrin." Unfortunately, acid-formation in dextrin was not determined in this study, and Winslow and Walker did not employ salicin or glycerol.

CHARACTERISTICS OF ORGANISMS FROM THE DIFFERENT SOURCES

When this study was begun (1915), motility and fermentation of dextrin and starch were regarded as of little significance and hence these tests were omitted. In the following year (1916) the possible significance of the reactions was realized and, as the cultures were still available, they were tested out. Motility was determined in a soft agar medium consisting of nutrient broth and 0.5% agar.

In Table 13 are shown the number and percentage of organisms giving positive reactions with the various tests. Glucose, galactose, mannitol, maltose, and lactose are fermented by all strains, with gas-production. Inulin is not fermented by any of the organisms, and gelatin is uniformly negative in 20 days at 20 C. Gas is formed from glycerol by 76.2%, from salicin by 66.1%, from raffinose by 50.7%, from sucrose by 48.7%, from dulcitol by 43.6%, from dextrin by 5.1%, and from starch by 4.5%. The Voges-Proskauer reaction is given by 5.8%, indol is produced by 91.1%, and 61.5% are motile.

Table 14 shows the characters of organisms isolated from different sources. Characters which are negative or positive for all strains are omitted.

Several things are evident. Organisms giving a positive Voges-Proskauer reaction or gas from dextrin and starch were obtained

TABLE 13

GAS-FORMATION AND OTHER CHARACTERISTICS OF BACILLUS-COLI-LIKE BACTERIA FROM VARIOUS ANIMALS AND SEWAGE

Character	Number Positive	Percentage Positive
Motility	96	61.5
Gelatin	0	0
Indol	142	91.1
Voges-Proskauer	9	5.8
Glucose	156	100
Galactose	156	100
Mannitol	156	100
Dulcitol	68	43.6
Glycerol	118	76.2
Maltose	156	100
Lactose	156	100
Sucrose	76	48.7
Raffinose	79	50.7
Salicin	102	66.1
Dextrin	8	5.1
Inulin	0	0
Starch	7	4.5

only from sewage. This must not be taken to mean that such organisms are entirely absent from the other sources, but it certainly indicates that they are extremely scarce in feces of the animals studied.

Salicin is fermented by 95% of the bovine strains, and by 8 (89%) of the 9 Voges-Proskauer-positive strains from sewage. Organisms

TABLE 14

MOTILITY AND OTHER REACTIONS OF BACILLUS-COLI-LIKE BACTERIA FROM DIFFERENT SOURCES

Source	Horse	Sheep	Cow	Pig	Man	Sewage	
						V. P. —	V. P. +
Number of strains	19	22	20	31	25	30	9
	Percentage of Positive Reactions						
Motility	100.0	77.3	80.0	93.7	32.0	20.0	11.1
Voges-Proskauer reaction	0.0	0.0	0.0	0.0	0.0	0.0	100.0
Indol	100.0	100.0	100.0	93.7	84.0	83.5	66.7
Sucrose	79.0	95.5	50.0	32.3	12.0	26.6	100.0
Raffinose	73.8	100.0	50.0	32.3	16.0	33.3	100.0
Dulcitol	68.5	50.0	50.0	42.0	20.0	43.3	33.3
Glycerol	84.3	62.0	95.0	74.2	64.0	70.0	88.9
Salicin	73.8	68.3	95.0	58.1	44.0	60.0	88.9
Dextrin	0.0	0.0	0.0	0.0	0.0	0.0	88.9
Starch	0.0	0.0	0.0	0.0	0.0	0.0	77.8

from other sources attack salicin less readily—horse 73.8%, sheep 68.3%, pig 58.1%, man 44%, and sewage (Voges-Proskauer-negative strains) 56.7%. This glucosid was used by MacConkey,[3] who did not regard its employment worth while for classification purposes. Recently (1914) Kligler[6] suggested that salicin displace dulcitol in

subdivision of the colon-bacillus group, but Rogers[7] questions the value of salicin in view of the very large number of his strains which attacked it. In this connection it might be well to point out that organisms studied by Rogers consisted of bovine strains, grain strains (probably Voges-Proskauer-positive organisms), and milk strains (which may be considered for the most part as a mixture of bovine and grain strains). In view of the results obtained here with bovine and Voges-Proskauer-positive strains, and by Kligler with Voges-Proskauer-positive strains, it would be expected that more than 90% of Rogers' cultures would attack salicin. It appears then that salicin-fermentation is somewhat correlated with the source.

Glycerol is also fermented by almost all the Voges-Proskauer-positive and bovine strains and less frequently by organisms from the other animals, but the difference is less marked than with salicin.

Dulcitol is only occasionally fermented by the human and Voges-Proskauer-positive strains, but there seems to be very little relation between dulcitol-fermentation and the animal source.

Indol-production is not correlated with the animal source.

In motility there is a marked contrast between the strains from horse, sheep, cow, and pig on the one hand, and those from man and sewage on the other. Less than one-third of the sewage and human strains are motile, as compared with more than four-fifths of the other animal strains. McWeeney[9] found nonmotile B. coli abundant in feces, and notes that Stocklin also had observed many nonmotile forms among fecal strains. Just what significance is to be attached to motility is hard to say at present, because so few bacteriologists determine this character in routine work. MacConkey, however, strongly advocates the test. As determined in the 0.5% agar medium the motility test is simple, quick, and not at all burdensome.

Sucrose and raffinose are so well correlated that a consideration of either will suffice for both. The Voges-Proskauer-positive and sheep strains are practically all sucrose-fermenters (100% and 95.5% respectively). Of the horse strains 79%, and of the organisms from the cow 50% form gas from sucrose; only 32.3% of strains from the pig, 26.6% of those from sewage (Voges-Proskauer-negative strains), and 12% of those from man form gas from sucrose. That such a small number of human strains attack sucrose is particularly interesting, and a review of the literature indicates that similar results have

[9] Cited by Prescott and Winslow, Elements of Water Bacteriology, 1913.

TABLE 15

FERMENTATION OF SUCROSE BY BACILLUS-COLI-LIKE BACTERIA FROM HUMAN FECES

Investigators	Number of Organisms Studied	Number of Sucrose Fermenters	Percentage of Sucrose Fermenters
Houston,[11] 1902-3	100	30	30
MacConkey,[3] 1905 and 1909	419	142	33.9
Ferreira,[12] Horta, Paredes, 1908	117	44	37.6
Winslow[8] and Walker, 1907	25	8	32
Howe,[10] 1912	540	324	60
Clemesha,[13] 1912	1200	348	29
Browne,[1] 1915	175	20	11.3
Levine,[4] 1916	25	3	12
Total	2601	919	35.3

been obtained by previous investigators. In Table 15 is shown the proportion of sucrose-fermenters obtained from human feces by different investigators. Howe[10] found 60% of 540 Bacillus-coli-like organisms to be sucrose fermenters, but the other investigators usually found twice as many nonfermenters as fermenters. Of 2601 cultures of human colon bacilli studied by various observers, at different times and in different countries, only 35.3% fermented sucrose.

In connection with the study reported here it should be noted that the number of human strains isolated is small, and that they were collected in the winter. Clemesha,[13] and also Browne,[1] call attention to "epidemics" of certain types of B. coli, and to seasonal variations. These phases need further investigation.

CONCLUSIONS

In studies on quantitative acid-production the average should be supplemented with a statement of its deviation measures; the unqualified average may lead to a misconception of the acid-producing properties of a group of organisms.

Quantitative acid-production in glucose, galactose, maltose, lactose, sucrose, raffinose, salicin, inulin, mannitol, dulcitol, and glycerol, is not a reliable index for differentiating colon-bacillus-like bacteria derived from pig, horse, sheep, cow, or man.

The MacConkey types are indistinguishable on the basis of quantitative acid-production in the fermentable carbohydrates, the alcohols, and the glucosid studied.

[10] Science, 1912, 35, p. 225.
[11] Suppl. to 32nd Ann. Rep. containing Rep. of Med. Officer for 1902-1903, p. 511.
[12] Arch. d. Real Inst. Bacteriol. Camara Pestana, 1908, 2, p. 153.
[13] Jour. Hyg., 1912, 12, p. 463.

The Voges-Proskauer-positive strains (aerogenes-cloacae group) form somewhat less acid from glucose, but more acid from maltose, sucrose, glycerol, and dulcitol, and possibly also from raffinose and salicin, than do the Voges-Proskauer-negative strains (colon-bacillus group).

Acid-formation should not be given precedence over gas-formation in studies on B. coli, for the acid may be masked by a secondary alkali-production. In general, however, acid-production is accompanied by gas-formation. With sucrose, dulcitol, and raffinose, acid-production and gas-formation are almost perfectly correlated. The correlation is less marked in the case of salicin, while the line of demarcation between gas-formers and nongas-formers, as indicated by quantitative acid-production from glycerol, is very indistinct.

Practically all Voges-Proskauer-positive and bovine strains attack salicin with liberation of gas. This glucosid is fermented less frequently by the organisms from pig, horse, sheep, man, and sewage

Gas is formed from sucrose as follows: Voges-Proskauer-positive (Voges-Proskauer-negative strains).
strains 100%, sheep 95.6%, horse 79%, cow 50%, pig 32.3%, sewage (Voges-Proskauer-negative strains) 26%, and human strains 12%.

Of 2601 human strains of B. coli studied by different investigators, in various countries and at different times, only 35.3% have been sucrose-fermenters.

Motility, as determined in semisolid nutrient agar, seems to be an important character. Only 32% of the human and 20% of the Voges-Proskauer-negative sewage strains are motile, as compared with 93.7% of pig, 80% of cow, 77.3% of sheep, and 100% of horse strains.